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CHOLYCYSTEIC ACID

By SIDNEY FREDRICK VELICK

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THE SYNTHESIS OF DICHOLYLCYSTINE AND CHOLYLCYSTEIC ACID*

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The mechanism by which taurocholic acid is formed in the body has not yet been established. It has been suggested that the taurine is derived from cystine by the series of reactions, cystine→cysteic acid→taurine, which has long been known to the organic chemist (1, 2). The possibility that taurine, thus formed, is conjugated directly with cholic acid is suggested by experiments in which simultaneous feeding of taurine and cholic acid to dogs increased the alcohol-soluble sulfur of the bile, whereas either taurine or cholic acid fed separately under suitable conditions did not alter the amount of this sulfur fraction or of the conjugated amino nitrogen in the bile (3, 4). It has also been observed that the alcohol-soluble sulfur of the bile increases after the administration of cystine and cholic acid together (3, 4).

If, in the living organism, taurine is derived from cystine, it is possible that the conversion of cystine to taurine may occur before the conjugation of the latter substance with cholic acid. The behavior of taurine when fed with cholic acid favors this mechanism. However, it is also possible that cystine is conjugated with cholic acid and the cholyrcystine thus formed is converted to taurocholic acid (cholyltaurine). Such a mechanism is supported by the experiments of Blum (5) who injected cystine into the vein of a dog and was able to obtain from the bile an alcohol-soluble substance containing labile sulfur and cholic

* A preliminary report of this investigation was presented before the Thirty-second annual meeting of the American Society of Biological Chemists at Baltimore, March 30–April 2, 1938 (Velick, S. F., and White, J., *Proc. Am. Soc. Biol. Chem., J. Biol. Chem.*, **123**, p. cxxiii (1938)).

478 Dicholycystine and Cholycysteic Acid

acid. These properties suggest the presence of dicholycystine or some similar substance in the bile.

The following series of reactions may represent the biological synthesis of taurocholic acid (cholytaurine): cholic acid + cystine $\rightarrow$ dicholycystine; dicholycystine $\rightarrow$ cholycysteic acid; choly-cysteic acid $\rightarrow$ cholytaurine.

In order to study this proposed mechanism, dicholycystine¹ and cholycysteic acid have been synthesized. Triformylcholic acid was converted to the acid chloride and coupled in chloroform solution with the dimethyl ester of cystine. Selective hydrolysis in dioxane converted the ester to the acid. The formyl groups were removed by the action of anhydrous ammonia in methyl alcohol and the product was isolated as the diammonium salt. Acidification with dilute hydrochloric acid produced the free acid. Cholycysteic acid was prepared by the oxidation of di(tri-formylcholy)-cystine dimethyl ester with bromine and subsequent removal of the formyl groups with sodium methylate. The cholycysteic acid was isolated as the sodium salt.

EXPERIMENTAL

Triformylcholic Acid—This compound was prepared by a method essentially the same as that of Cortese and Baumann (7). 50 gm. of cholic acid (Hoffmann-La Roche), m.p. 196°, were dissolved in 100 cc. of redistilled formic acid and the mixture was heated for 6 hours at 60° in a glass-stoppered flask. The clear yellowish reaction mixture was poured slowly and with vigorous stirring into several volumes of distilled water containing cracked ice. The white granular precipitate which formed was filtered by suction, dissolved in 500 cc. of hot 95 per cent alcohol, and 600 cc. of boiling water were added gradually. After standing 5 hours at room temperature, the crystals were filtered off and recrystallized again. 50 gm. were obtained. Yield, 83 per cent. M.p. 206°.

¹ After the completion of this work, the synthesis of cystocholic acid (dicholycystine) was reported from Sobotka's laboratory by Holzmann (6). Beyond the statement that the synthesis was carried out by the procedure of Curtius, no details are available. We also prepared small amounts of dicholycystine by the azide method, but abandoned this method, since better yields were obtained by the more convenient procedure described in this paper.

Triformylcholy Chloride—The method of Cortese and Baumann (8) was employed. It was necessary to distil the thionyl chloride from quinoline and then from linseed oil in a moisture-free chamber immediately before each run. Unless these precautions were taken, the product became highly colored.

Dimethyl Ester of Di(Triformylcholy)-Cystine—A solution of 10 gm. of freshly prepared triformylcholy chloride dissolved in 100 cc. of dry chloroform was added gradually to twice the equivalent amount of the dimethyl ester of cystine in 70 cc. of chloroform. The solution was allowed to stand for 3 hours at room temperature. The precipitated ester hydrochloride of cystine was removed by filtration and the filtrate was extracted several times with 10 per cent hydrochloric acid, then with 5 per cent sodium carbonate, and finally with water. The chloroform solution, after extraction, was dried over sodium sulfate and the chloroform was removed *in vacuo* on a water bath at 60°. The residue was crystallized from hot capryl alcohol. The needles melted at 88–90°. Yield, 70 per cent.

$C_{62}H_{94}O_{18}N_2S_2$. Calculated, N 2.29, S 5.25; found, N 2.16, S 5.08

Diammonium Salt of Dicholylcystine—The dimethyl ester of di(triformylcholy)-cystine was dissolved in 10 times its weight of dioxane, 8 equivalents of N sodium hydroxide were added, and the solution was shaken in a stoppered flask for 20 minutes. An amount of N hydrochloric acid equivalent to the alkali used was added and the dioxane was distilled off *in vacuo*. This procedure removed the methyl groups and some of the protecting formyl groups. Without further purification, 2 gm. of the dried product were dissolved in 20 cc. of dry ethyl alcohol that had been saturated with anhydrous ammonia and the solution was allowed to stand 16 hours at room temperature in a glass-stoppered flask. The ammonia and the alcohol were then distilled off *in vacuo*. The residue was extracted with ether and dried *in vacuo* over phosphorus pentoxide. Yield, 87 per cent.

$C_{54}H_{94}O_{12}N_4S_2$. Calculated, N 5.31, S 6.07; found, N 5.09, S 6.13

Dicholylcystine—The clear colorless water solution of the above diammonium salt was precipitated with 10 per cent hydrochloric acid. The white, powdery precipitate was filtered by suction,

480 Dicholyleystine and Cholyleysteic Acid

washed with water, and dried *in vacuo* over phosphorus pentoxide. The yield was almost quantitative.

$C_{64}H_{88}O_{12}N_2S_2$. Calculated, N 2.74, S 6.26; found, N 2.81, S 6.17

Methyl Ester of Triformylcholyleysteic Acid—2 gm. of the dimethyl ester of di(triformylcholy)-cystine were pulverized to a fine powder and suspended in 50 cc. of water, and the mixture was stirred mechanically. 1.29 gm. of bromine were added in small portions as rapidly as the color of the bromine disappeared. The solution was filtered from a trace of unoxidized ester, extracted with ether, and shaken with a slight excess of moist silver oxide. The solution was filtered rapidly and the filtrate was treated with hydrogen sulfide to remove the excess silver. The silver sulfide could not be readily filtered off, as it was present in colloidal form. However, by concentrating this colloidal solution to dryness *in vacuo* on a water bath, and by subsequently extracting with dry methyl alcohol, a clear solution free from silver was obtained. The straw-colored extracts were decolorized with carbex E and the alcohol was removed in a vacuum desiccator over sulfuric acid. The colorless product was dried *in vacuo*. Yield, 77 per cent.

$C_{31}H_{47}O_{12}NS$. Calculated, N 2.13, S 4.87; found, N 2.10, S 4.90

It is not necessary to isolate this compound for the succeeding steps inasmuch as the dry methyl alcohol solution can be used directly.

Ammonium 26-Carbamyltaurocholate (β -Carbamyltaurocholate)—A solution of 1 gm. of triformylcholyleysteic acid (methyl ester) in 20 cc. of anhydrous methyl alcohol was saturated with dry ammonia and was allowed to stand for 16 hours at room temperature. The alcohol and excess ammonia were removed *in vacuo*. The residue was dissolved in methyl alcohol, decolorized by boiling with a little carbex E, filtered, and 3 volumes of dry ether were added. The precipitate was filtered off and dried in a vacuum desiccator.

$C_{27}H_{49}O_8N_3S$. Calculated, N 7.30, S 5.55; found, N 7.39, S 5.52

This derivative was prepared as a confirmatory compound for analysis.

Disodium Salt of Cholylcysteic Acid—1.55 gm. of the methyl ester of triformylcholylcysteic acid (from 2 gm. of the dimethyl ester of di(triformylcholyl)-cystine were dissolved in 10 cc. of dry methyl alcohol and a solution of 0.72 gm. of sodium in 10 cc. of dry methyl alcohol was added. After standing at room temperature for an hour, the finely divided white precipitate was filtered off and washed with dry alcohol and ether. It was then dissolved in a hot solution of 5 cc. of absolute methyl alcohol and 2 cc. of water. 8 cc. of absolute methyl alcohol were added and the solution was chilled overnight. The supernatant liquid was decanted from the colorless oil which separated. The oil was washed with dry alcohol, was covered with dry ether, and was allowed to stand 6 hours. The solid mass was pulverized, filtered, and dried *in vacuo*.

$C_{27}H_{43}O_8NSNa_2$. Calculated, N 2.32, Na 7.6; found, N 2.25, Na 7.3

The biological studies of these compounds will be reported at a later date.

SUMMARY

The peptide, dicholylcystine, has been synthesized and its oxidation to cholylcysteic acid is described. These compounds are of interest in view of the suggestion that the conjugation of cholic acid with cystine may occur *in vivo* as a reaction in the biological synthesis of taurocholic acid (5, 9).

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